Intra-burst firing characteristics as network state parameters

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Abstract

Network bursts are the dominant type of activity patterns that neuronal networks in culture spontaneously produce. We show that the spatio-temporal structure of bursts, while not deterministic, is statistically stable over a period of several hours. We use the instantaneous array-wide spiking rate during bursts to compare bursts with each other. The statistical structure is thought to reflect the functional connectivity, or state, of the network. The analysis presented can be useful to study (induced) plasticity.

1 Introduction

In our group we are aiming to demonstrate learning and memory capabilities of cultured networks of cortical neurons. A first step is to identify parameters that accurately describe changes in the network due to learning. Usually, such parameters are calculated from the responses to test-stimuli before and after a learning experiment [1,2,3]. We propose that parameters should be calculated from the spontaneous activity before and after a learning experiment, as the applying of test-stimuli itself may alter the network (see also [4]).

Spontaneous activity is generally dominated by *network bursts*; periods in which the spiking activity is very high, as compared to the nominal level. Consequently, most of the data collected from spontaneous activity originates from bursts. Bursts are present throughout the period that we measured (from 7 to 61 DIV), although the make-up of bursts change with age [5,6]. Due to the fact that the burst appearance changes with age but is quite stable over a period of hours, any parameters extracted from bursts should have a natural time-base that would be useful to observe changes due to stimulation algorithms.

Spontaneous activity patterns, and particularly network bursting events, are closely related to the development of synaptic connections. In early stages of development (DIV4 to DIV14), this can be attributed to new outgrowth of synapses. After this period, a decline in number of synapses occurs as the culture enters a mature state. The spontaneous activity associated with each stage maturation is markedly different, thus showing the dependence of spontaneous activity on the physical network structure. However, the formation and elimination of synapses is also dependent on the electrical activity, as shown in [7].

2 Materials & methods

2.1 Culturing

Cortical neurons are obtained from newborn E18 Wistar rats by trituration and chemical dissociation using trypsin. The cells were plated in a concentration of 1M cells/ml, and allowed to adhere for 4 hours. The MEA's were coated with polyethylene-imine (PEI) to increase adhesion. The resulting monolayer had a density of ~5000 cells/mm^2. The cultures were stored in an incubator at 37 ºC, at a CO_2 concentration of 5 % and near 100% humidity, in R12 medium (DMEM/HAM's F12, Gibco) without serum. The medium was entirely changed 2 times a week.

2.2 Measurement setup

We use commercially available MEA's from MultiChannel Systems (MCS) with 60 Titanium-Nitride electrodes in a square grid. The inter-electrode distance is 100 µm, and the diameter of the electrodes is 10 µm.

Signals were measured using the MC1060BC preamplifier and FA60s filter amplifier (both MCS) to prepare the signals for AD-conversion. Amplification is 1000 times in a range from 100 Hz to 6000 Hz. A 6024E data-acquisition card (National Instruments, Austin, TX) is used to record all 60 channels at 16 kHz. Custom-made Labview (National instruments, Austin, TX) programs are used to control the data acquisition. During the experiments, the temperature was controlled at 36.0 ºC, using a TC01 (MCS) temperature controller.

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2.3 Data analysis

Spikes were detected online using a threshold detection algorithm. The threshold was set at 5 to 6 times the RMS noise level, which was continually monitored and was typically in the range of 3 to 5 μV_{RMS}. The spikes were validated offline using the algorithm described by [8].

Network bursts were detected by analysing the Array-Wide Spiking Rate (AWSR, the sum of activity over all electrodes) in bins of 100 ms. If a threshold was crossed, adjacent bins were added if it had more spikes than 20% of the maximum. The threshold was set at 2 times the number of active electrodes (i.e. having a firing rate > 0.1 Hz), with a minimum of 10.

The instantaneous AWSR during a burst was estimated by convolving spike-occurrences with a Gaussian function. A standard deviation of 5 ms was wide enough in most cases to obtain a smooth function near the peak AWSR. This is necesserary for a good estimation of the location of the peak AWSR.

We investigated the changes in these *burst profiles* over time by aligning them to their peak AWSR. To this end, the profiles were grouped over 1 hour of measurement. Differences in and between these sets

$$E(a,b) = \frac{\sigma^2_{a-b}}{\sigma^2_b}$$

are calculated using the following equation:
where a is an individual profile and b is an average taken over an hour of measurement. The normalisation is only to σ_b, so that the measure is not symmetrical.

In addition, a sufficient amount of aligned bursts yielded enough data to calculate the contribution of each recording site. The instantaneous spike rate for each site was also calculated by convolving all spikes with a gaussian.

3 Results

3.1 Hour-to hour variation

The burst profiles, calculated over a period of 1 hour, generally show little variation (figure 1).

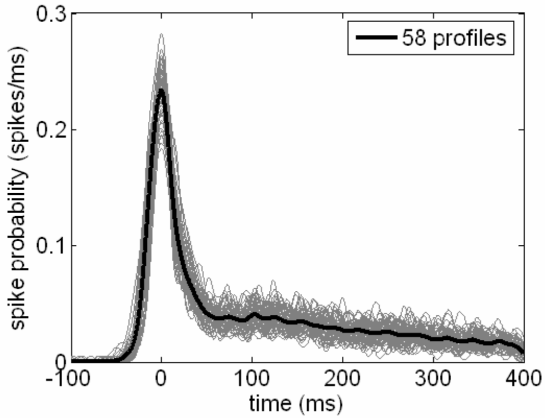

Fig. 1 Profiles aligned during 1 hour of measurement (DIV 11). Corresponds to the 2nd hour in figures 2 and 3.

The differences in subsequent hours are shown in figures 2 and 3. Figure 2 shows that in the last two hours of measurement the peak AWSR is lower than in the 6 hours before which are very similar to each other.

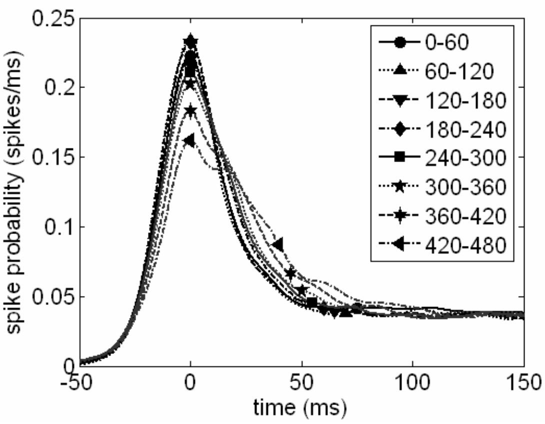

Fig. 2 Profiles in subsequent hours of measurement.

In figure 3 the normalized error E is calculated between all 8 hours (blocks) of a measurement and is displayed as shades of grey. Every row presents normalised errors with the same block as a reference. The variation within blocks are thus on the diagonal. The normalised errors on the diagonal are usually the smallest, indicating that the profiles are correlated in time.

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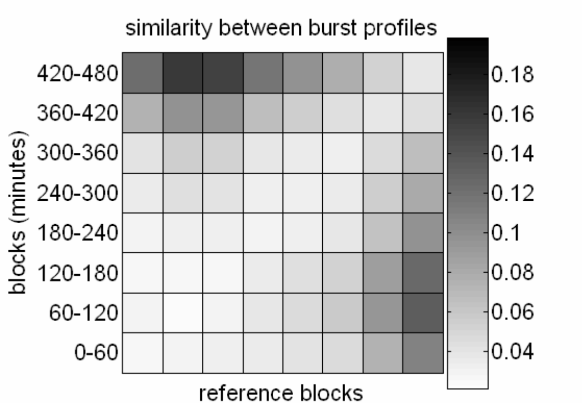

Fig. 3 Similarity between burst profiles.

The relatively slow changes over the period of hours indicate an underlying probabilistic structure in the AWSR during bursts. This is revealed in more detail by showing the contributions of individual sites (figure 4). The phases in which individual sites contribute most to a burst differs from site to site, indicating a kind of statistical spatio-temporal ordering in the makeup of bursts.

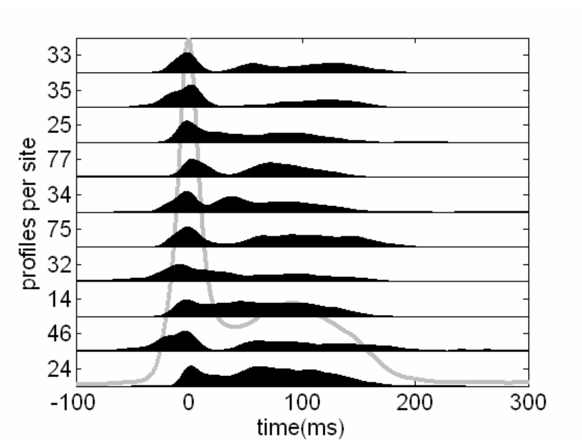

Fig. 4 Profiles of the 10 most active sites. The average burst profile is shown in grey.

3.2 Day-to day variation

Over a period of days, the shapes are distinguishably different (figure 5). On DIV11, the bursts have a single peak and a long tail. By DIV13, this has changed into bursts with a distinct second phase of firing. Over the next days the second phase declines and on DIV16 it has disappeared entirely.

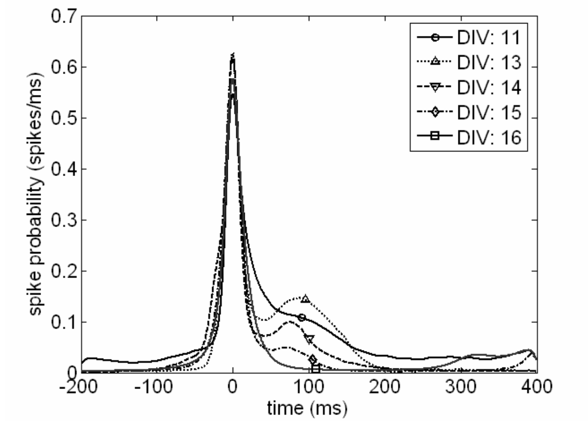

fig. 5 Average burst profiles per day of measurement.

The differences in shape are also visible in figure 6, where the normalised errors are shown for the same measurements as shown in figure 5. Here, 2 hours of each measurement was divided in 4 blocks of 30 minutes.

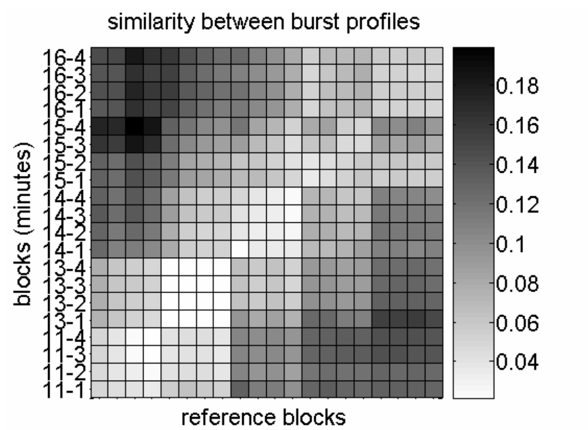

Fig. 6 Similarity in- and between measurements. Each measurement is divided in 4 blocks of 30 minutes.

The partitioning in 4x4 blocks indicates that the correlation between blocks on a particular day are correlated among themselves more than to blocks on any other day.

4 Discussion

The apparent structure in the burst profiles result from the relationships between individual recording sites, and thus also on the connectivity in the neural network.

The burst profiles prove to be stable over a period of one hour, and gradually change their shape over several hours, as has also been suggested in [5]. The day-to-day changes in burst profiles may be the result of these gradual changes, thereby suggesting an intrinsically changing network. However, they can also be the result of putting the cultures back in the stove. The spike envelopes per recording site offer more detailed descriptions of the network state than the burst profiles. This may however be the amount of detail required to reveal the changes made during learning experiments.

A subsequent refinement can be made by identifying distinct subgroups of bursts, as has been suggested in [9]. This may be necessary for the complex firing patterns seen in cultures in a mature state.

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